

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Bernd STAHL et al. Confirmation No: 4218  
Appl. No. : 10/502,059  
Filed : August 2, 2004  
Title : CYCLOGLYCANS SUITABLE TO INHIBIT MAMMALIAN INFECTION  
TC/A.U. : 1609  
Examiner : J.S. Lau  
Docket No.: : STA3007/REF/PAD  
Customer No: : 23364

Declaration Under 37 CFR 51.132

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

I, *Bernd Stahl*, hereby declare as follows:

My relevant background and experience are set forth on the attached c.v.

I have read the outstanding Official Action in the above-identified application and the references cited and applied therein.

I make this declaration in support of the present application, and to provide evidence in rebuttal to the contentions set forth in the outstanding Official Action. In particular, I declare that the disclosure of the above-identified application does enable one skilled in the art to make and use the invention as set forth in the claims.

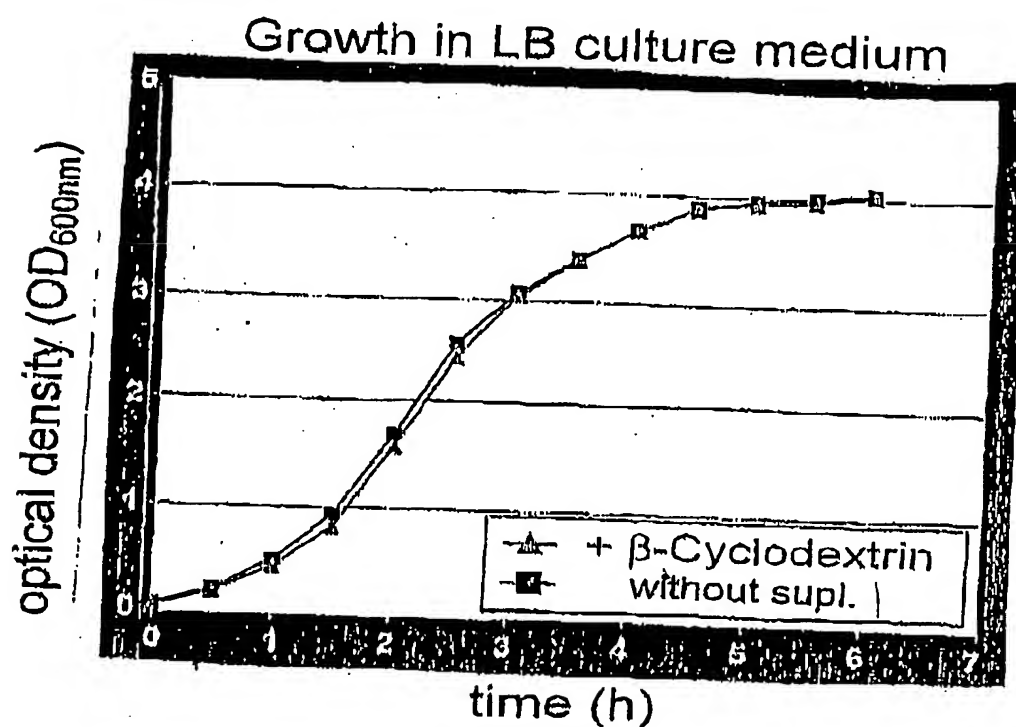
In support of my position, several experiments have been conducted that show by the administration of cycloglycans in accordance with the claimed invention to cells can successfully inhibit the invasion and infection of the cells by a variety of pathogenic, intracellular bacteria.

The adhesion of pathogenic organisms to a cell is the first step and an essential prerequisite for infecting a cell. The interaction between pathogenic bacteria and mammalian cells is controlled by a ligand-receptor relationship. The claimed invention is based on the discovery that administering cycloglycans in accordance with the claimed

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invention can interfere with this relationship and inhibit pathogenic, intracellular bacteria from invading a mammalian cell. One skilled in the art would have understood that the claimed method has not necessarily been developed to inhibit the invasion and infection of a particular pathogen, but to take advantage of this ligand-receptor relationship to inhibit the invasion and infection of a variety of pathogenic, intracellular bacteria.

The growth of bacterium EIEC4608-58 in LB broth with and without the addition of  $\beta$ -cyclodextrin is shown as follows:

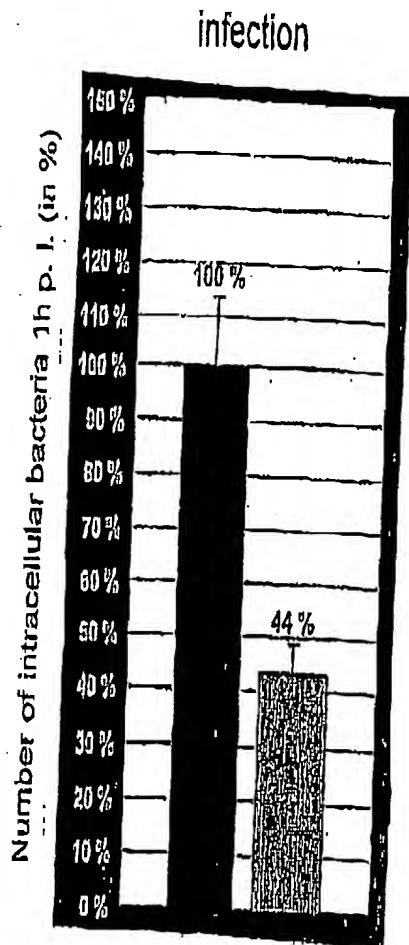


• no influence of the bacterial replication

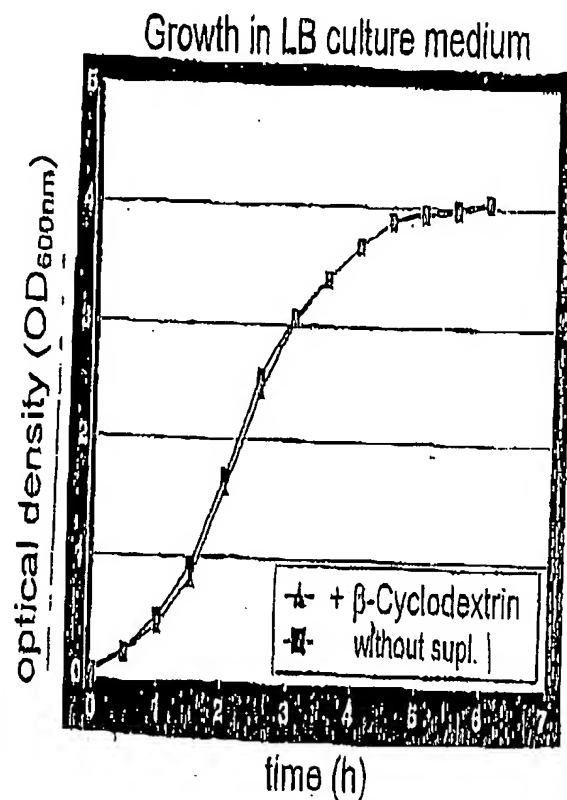
Applicants respectfully submit that the growth analysis with EIEC4608-58 shows that the growth of the bacteria is not influenced by the addition of  $\beta$ -Cyclodextrin.

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However, in vitro infection studies and growth analysis with *S. typhimurium* 14028 have also been conducted. The results are as follows:



without suppl.  
 $\beta$ -cyclodextrin



- reduced uptake of bacteria
- no influence on the bacterial replication

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The results show that the addition of  $\beta$ -Cyclodextrin inhibits the uptake of *S. typhimurium* 14028 to inhibit infections, yet does not inhibit the growth of the cell or the replication of bacteria.

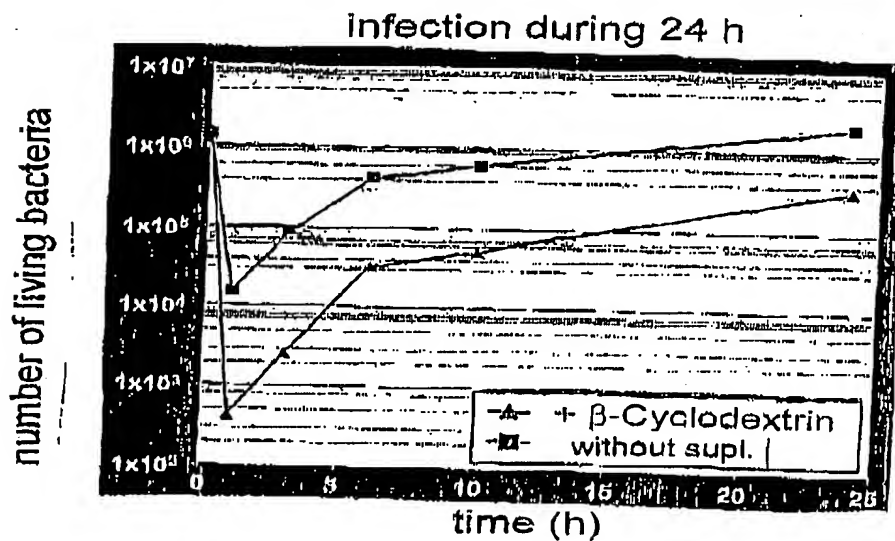
This is confirmed with the graph "Abstract of the results obtained with  $\beta$ -Cyclodextrin" and "In-vitro infection studies with EIEC 4608-58" shown as follows:

## Abstract of the results obtained with $\beta$ -cyclodextrin

Cell culture	$\beta$ -cyclodextrin reduces the uptake of EIEC 4608-58 (3%) as well as of <i>S. typhimurium</i> 14028 (44%) in caco-2 enterocytes
Laboratory culture	$\beta$ -cyclodextrin does not influence the bacterial growth of EIEC 4608-58 and of <i>S. typhimurium</i> 14028
2D-gels	$\beta$ -cyclodextrin does not influence the protein expression of EIEC 4608-58 as well as of <i>S. typhimurium</i> 14028

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### IN-VITRO INFECTION STUDIES WITH EIEC 4608-58

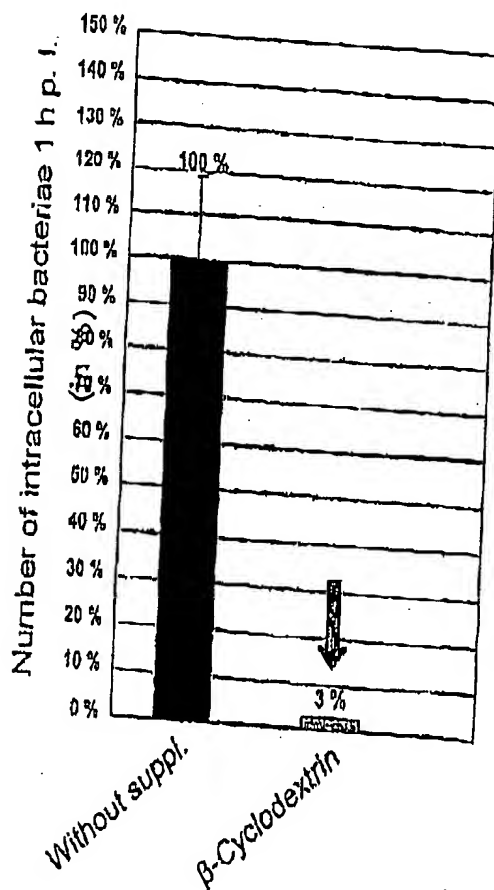


- reduced uptake of bacteria
- no influence on the intracellular replication

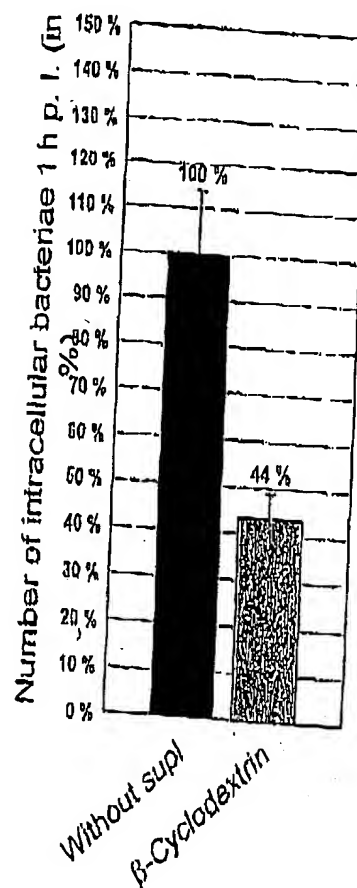
Studies have also been conducted that show that the invasion of bacteria in Caco 2-cells is inhibited with the administration of a cyclodextrin in accordance with the claimed invention as follows:

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Invasion of *Escherichia coli*,  
strain EIEC 4608-58



Invasion of *Salmonella*  
typhimurium, strain 14028



Applicants have also performed tests in which J774-cells were infected with *L. monocytogenes* in the presence of several sugars. These results are summarized as follows:

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Example 1:

Infection of J774 cells with *L. monocytogenes* in the presence of several sugars/saccharides

Percentage of viable intra cellular bacteria isolated one hour after infection of J774-enterocytes in the presence of different sugars, based on the untreated control. Eukaryotic cells were pre-incubated one hour with the sugars mentioned, infected with *Listeria monocytogenes* strain 14028 in the presence of the sugar for a period of 5 minutes, washed, incubated with Gentamicin for one hour and finally lysed and plated.

Results

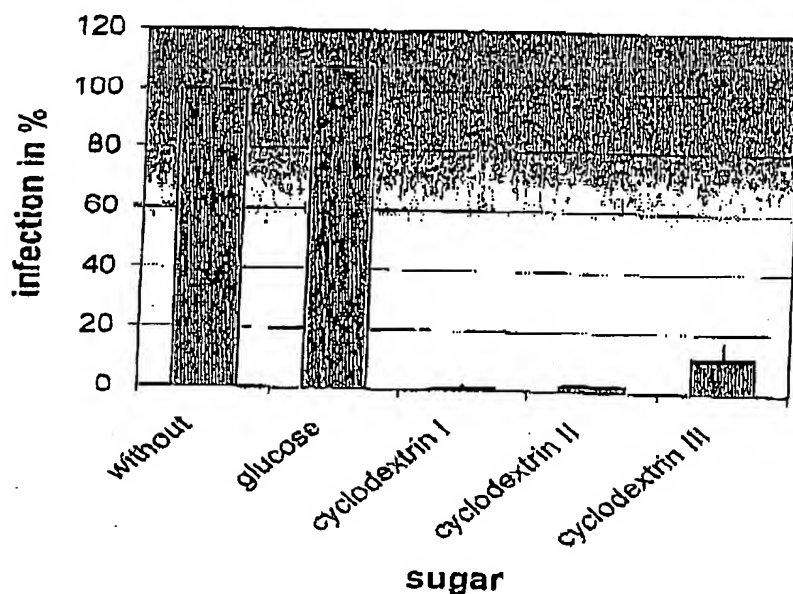
Sugar	Infection
Without	100 %
Glucose	105 %
Cyclodextrin I	1 %
Cyclodextrin II	2 %
Cyclodextrin III	13 %

The infection with the cyclodextrins is only 1%, 2% or 13% respectively, with the addition of a cyclodextrin in accordance with the claimed invention. This stands in contrast with the control, wherein the corresponding infection without the addition of sugar was 100%. In the presence of glucose, the corresponding rate of infection was 105%.

The results are as follows:

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**Infection of J774 cells with  
*L. monocytogenes* in the presence of  
different sugars**



Thus, the results show that the claimed method is effective for inhibiting the invasion and infection of several different types of cells from a variety of pathogenic, intracellular bacteria. It is believed to be apparent that administering a cyclodextrin in accordance with the claimed invention to a mammalian cell can inhibit the invasion and infection of the cells from a variety of pathogenic, intracellular bacteria. Thus, I declare that the disclosure of the above-identified application does enable one skilled in the art to make and use the invention as set forth in the claims.



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I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-captioned patent.

By: Bernd Stahl  
BERND STAHL

Date: 12.1.09